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Specification and Drawing, as originally filed, with Application for Patent Serial No: **2,327,628**, on December 5, 2000, by **VASOGEN IRELAND LIMITED**, for "Deprotection of Malignant Cells"

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ABSTRACT OF THE DISCLOSURE

A process of treating malignant cells in a mammalian patient to render them more susceptible to apoptosis, comprises administering to the patient harboring said malignant cells stressed blood cells which have been extracorporeally subjected to at least one stressor selected from oxidative stress and ultraviolet radiation.

DEPROTECTION OF MALIGNANT CELLS

Field of the Invention

This invention relates to treatment of malignancies in mammalian patients, and compositions useful therein. More specifically, it relates to methods for enhancing the effectiveness of chemotherapeutic and radiative treatments for inhibiting malignant cell proliferation and for destruction of malignant cells.

Background of the Invention

It is known that certain types of malignant cells express cytokines, during their proliferation. Some of these cytokines exert a protective effect on the malignant cells and serve to protect them from the destructive effects of chemotherapeutic agents such as anti-cancer drugs, and externally applied radiation. The effectiveness of cancer treatments, both chemotherapy and radiation, are often significantly limited by the protective effects of these cytokines.

For example in chronic lymphocytic leukemia, the tumor cell is a CD5+ B cell, which secretes the cytokine interferon γ (IFN-γ) as a protectant. Chronic lymphocytic leukemia is believed to progress, at least in part, as a result of the blocking of apoptosis by the body's regular defence mechanisms due to the presence of excess amounts of IFN-γ. The presence of this protective cytokine INF-γ significantly hampers attempts to treat chronic lymphocytic leukemias and similar malignancies with apoptosis inducing therapies such as administration of chemotherapeutic agents which generally act to eliminate malignant cells through apoptosis, or radiation, which generally acts to cause DNA damage and thereby induce apoptosis in rapidly dividing cells such as tumour cells. See Zaki, M. et.al., Leuk. Res., 2000 Jul; 24(7): 611-21.

Summary of the Invention

The present invention provides a process of treating a patient

suffering from a malignancy, which comprises administering to the patient stressed blood cells which are effective in down-regulating the expression of cytokines which exert a protective effect on the malignant cells. Accordingly, the protectant effect of the expressed cytokine is diminished or lost, rendering the malignant cells more susceptible to apoptosis and elimination as a result thereof, either by the body's own natural processes or by externally applied treatments such as chemotherapy or radiation techniques. The process involves introducing into the patient's blood or tissue, compatible mammalian blood cells which have been extracorporeally stressed by subjection to an oxidative stress and ultraviolet radiation. On introduction of these stressed blood cells, they have the effect of reducing the secretion of protective INF-γ, so that apoptosis such as that resulting from simultaneous or subsequent chemotherapy or radiation therapy is rendered more effective in destroying the malignant cells.

Thus according to the present invention, there is provided a process of treating malignant cells in a mammalian patient to render them more susceptible to apoptosis, which comprises administering to the patient harboring said malignant cells stressed blood cells which have been extracorporeally subjected to at least one stressor selected from oxidative stress and ultraviolet radiation.

According to another aspect of the present invention, there is provided a process of treating malignant cells in a mammalian patient to render them more susceptible to inhibition or destruction by chemotherapeutic agents or radiation therapy, which comprises administering to the patient harboring said malignant cells stressed blood cells which have been extracorporeally subjected to at least one stressor selected from oxidative stress and ultraviolet radiation.

According to another aspect of the invention, there is provided a process of inhibiting expression of cytoprotective cytokines, such as IFN-γ, in cells in a mammalian body, which comprises administering to the mammalian body blood components which have been extracorporeally subjected to at least one stressor selected from oxidative stress and ultraviolet radiation.

Brief reference to the Drawings.

The accompanying Figure of drawings is a presentation of the experimental results obtained according to the specific Example described below.

Description of the Preferred Embodiments.

The source of the stressed blood cells for use in the present invention is preferably the patient's own blood, i.e. an aliquot of autologous blood, or a cellular fraction thereof.

The terms "aliquot", "aliquot of blood" or similar terms used herein include whole blood, separated cellular fractions of the blood including platelets, separated non-cellular fractions of the blood including plasma, plasma components and combinations thereof. Preferably, in human patients, the volume of the aliquot is up to about 400 ml, preferably from about 0.1 to about 100 ml, more preferably from about 1 to about 15 ml, even more preferably from about 8 to about 12 ml, and most preferably about 10 ml. The effect of the stressor or the combination of stressors is to modify the blood, and/or the cellular or non-cellular fractions thereof, contained in the aliquot. The modified aliquot is then re-introduced into the subject_s body by any suitable method, most preferably intramuscular injection, but also including subcutaneous injection, intraperitoneal injection, intra-arterial injection, intravenous injection and oral administration, following which it causes decrease in the expression of one or more of the protective cytokines expressed by a malignant cell, e.g. INF-γ from malignant CD5+ B cells in lymphocytic leukemia.

According to a preferred process of the present invention, an aliquot of blood is extracted from the human patient, and the aliquot of blood is treated ex vivo, simultaneously or sequentially, with the aforementioned stressors. Then it is injected back into the same subject. Preferably a combination of both of the

aforementioned stressors is used.

Preferably also, the aliquot of blood is in addition subjected to mechanical stress. Such mechanical stress is suitably that applied to the aliquot of blood by extraction of the blood aliquot through a conventional blood extraction needle, or a substantially equivalent mechanical stress, applied shortly before the other chosen stressors are applied to the blood aliquot. This mechanical stress may be supplemented by the mechanical stress exerted on the blood aliquot by bubbling gases through it, such as ozone/oxygen mixtures, as described below. Optionally also, a temperature stressor may be applied to the blood aliquot, simultaneously or sequentially with the other stressors, i.e. a temperature at, above or below body temperature.

The optionally applied temperature stressor either warms the aliquot being treated to a temperature above normal body temperature or cools the aliquot below normal body temperature. The temperature is selected so that the temperature stressor does not cause excessive hemolysis in the blood contained in the aliquot and so that, when the treated aliquot is injected into a subject, the desired effect will be achieved, without development of significant adverse side effects. Preferably, the temperature stressor is applied so that the temperature of all or a part of the aliquot is up to about 55°C, and more preferably in the range of from about -5°C to about 55°C.

In some preferred embodiments of the invention, the temperature of the aliquot is raised above normal body temperature, such that the mean temperature of the aliquot does not exceed a temperature of about 55°C, more preferably from about 40°C to about 50°C, even more preferably from about 40°C to about 44°C, and most preferably about 42.5 ± 1°C.

In other preferred embodiments, the aliquot is cooled below normal body temperature such that the mean temperature of the aliquot is within the range of from about 4°C to about 36.5°C, more preferably from about 10°C to about

30°C, and even more preferably from about 15°C to about 25°C.

The oxidative environment stressor can be the application to the aliquot of solid, liquid or gaseous oxidizing agents. Preferably, it involves exposing the aliquot to a mixture of medical grade oxygen and ozone gas, most preferably by applying to the aliquot medical grade oxygen gas having ozone as a component therein. The ozone content of the gas stream and the flow rate of the gas stream are preferably selected such that the amount of ozone introduced to the blood aliquot, either on its own or in combination with one of the other stressors, does not give rise to excessive levels of cell damage, and so that, when the treated aliquot is injected into a subject, the desired effect will be achieved, without development of significant adverse side effects. Suitably, the gas stream has an ozone content of up to about 300 μg/ml, preferably up to about 100 μg/ml, more preferably about 30 μg/ml, even more preferably up to about 20 μg/ml, particularly preferably from about 10 μg/ml to about 20 μg/ml, and most preferably about 14.5 ± 1.0μ g/ml. The gas stream is suitably supplied to the aliquot at a rate of up to about 2.0 litres/min, preferably up to about 0.5 litres/min, more preferably up to about 0.4 litres/min, even more preferably up to about 0.33 litres/min, and most preferably about 0.24 ± 0.024 litres/min. The lower limit of the flow rate of the gas stream is preferably not lower than 0.01 litres/min, more preferably not lower than 0.1 litres/min, and even more preferably not lower than 0.2 litres/min, all rates at STP.

The ultraviolet light stressor is suitably applied by irradiating the aliquot under treatment from a source of UV light. Preferred UV sources are UV lamps emitting UV-C band wavelengths, i.e. at wavelengths shorter than about 280 nm. Ultraviolet light corresponding to standard UV-A (wavelengths from about 315 to about 400 nm) and UV-B (wavelengths from about 280 to about 315) sources can also be used. As in the case of the oxidative stressor, the UV dose should be selected, on its own or in combination of the other chosen stressor(s), so that excessive amounts of cell damage do not occur, and so that, when the treated aliquot is injected into a subject, the desired effect will be achieved. For example, an appropriate dosage of such UV light, can be obtained from up to eight lamps

arranged to be exposed to the sample container holding the aliquot, operated at an intensity to deliver a total UV light energy at 253.7 nm at the surface of the blood of from about 0.025 to about 10 joules/cm^2, preferably from about 0.1 to about 3.0 joules/cm^2. Such a treatment, applied in combination with the oxidative environment stressor, provides a modified blood aliquot which is ready for injection into the subject.

It is preferred to subject the aliquot to the oxidative environment stressor, the UV light stressor and the temperature stressor simultaneously, following the subjection of the aliquot to the mechanical stress, e.g. by extraction of the blood from the patient. Thus, the aliquot may be maintained at a predetermined temperature above or below body temperature while the oxygen/ozone gas mixture is applied thereto and while it is irradiated with ultraviolet light.

The time for which the aliquot is subjected to the stressors is normally within the time range of from about 0.5 minutes up to about 60 minutes. The time depends to some extent upon the chosen combination of stressors. When UV light is used, the intensity of the UV light may affect the preferred time. The chosen temperature level may also affect the preferred time. When oxidative environment in the form of a gaseous mixture of oxygen and ozone applied to the aliquot is chosen as one of the two stressors, the concentration of the oxidizing agent and the rate at which it is supplied to the aliquot may affect the preferred temperature. Some experimentation, well within the ordinary skill of the art, to establish optimum times may be necessary on the part of the operator, once the other stressor levels have been set. Under most stressor conditions, preferred times will be in the approximate range of from about 2 to about 5 minutes, more preferably about 3 minutes. The starting blood temperature, and the rate at which it can be warmed or cooled to a predetermined temperature, tends to vary from subject to subject. Warming is suitably by use of one or more infrared lamps placed adjacent to the aliquot container. Other methods of warming can also be adopted.

As noted, it is preferred to subject the aliquot of blood to a mechanical stressor, as well as the chosen stressor(s) discussed above. Extraction of the blood aliquot from the patient through an injection needle constitutes the most convenient way of obtaining the aliquot for further extracorporeal treatment, and this extraction procedure imparts a suitable mechanical stress to the blood aliquot. The mechanical stressor may be supplemented by subsequent processing, for example the additional mechanical shear stress caused by bubbling as the oxidative stressor is applied.

In the practice of the preferred process of the present invention, the blood aliquot may be treated with the heat, UV light and oxidative environment stressors using an apparatus of the type described in aforementioned U.S. Patent No. 4,968,483 to Mueller. The aliquot is placed in a suitable, sterile container, which is fitted into the machine. A UV-permeable container is used and the UV lamps are switched on for a fixed period before the other stressor is applied, to allow the output of the UV lamps to stabilize. When a temperature stressor is used combination, the UV lamps are typically on while the temperature of the aliquot is adjusted to the predetermined value, e.g. 42.5 $\pm$ 1°C. Four UV lamps are suitably used, placed around the container.

In the preferred method of the invention, a mammalian patient undergoing or awaiting chemotherapy or radiation for a malignancy such as a lymphoma is given one or more courses of treatments, each course of treatment comprising the administration to a mammalian subject of one or more (e.g. one to six) aliquots of mammalian blood modified as discussed above. No more than one aliquot of modified blood should be administered to the subject per day.

Although it may be sufficient to administer only one course of treatment as described above to the subject, it may be preferred in some circumstances to administer more than one course of treatment, or to follow the above-described course of treatment by periodic "booster" treatments, if necessary, to maintain the desired effects of the present invention. The course of

treatments should continue as long as the patient is undergoing chemotherapy or radiation or other treatment designed to attack the malignant cells.

The invention is further illustrated and described with reference to the following specific example, comprising animal studies conducted in an approved manner.

EXAMPLE

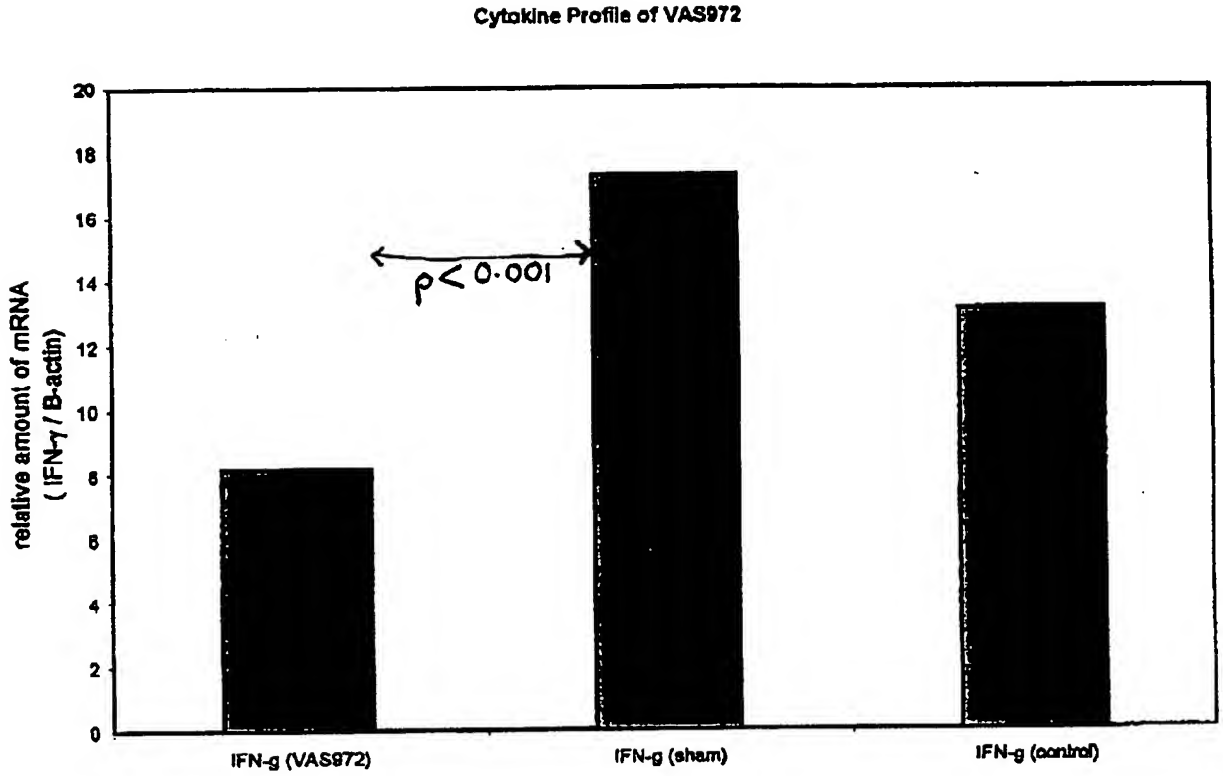
Whole blood was obtained from Balb/c mice, by extraction from a main artery through an injection needle, and treated with an anti-coagulant. An aliquot of this was subjected to the process described below, to obtain treated blood. The remainder was left untreated, for use in control experiments. Since these mice are genetically identical, the administration of the treated blood to others of the group is equivalent to administration of the treated blood to the donor animal.

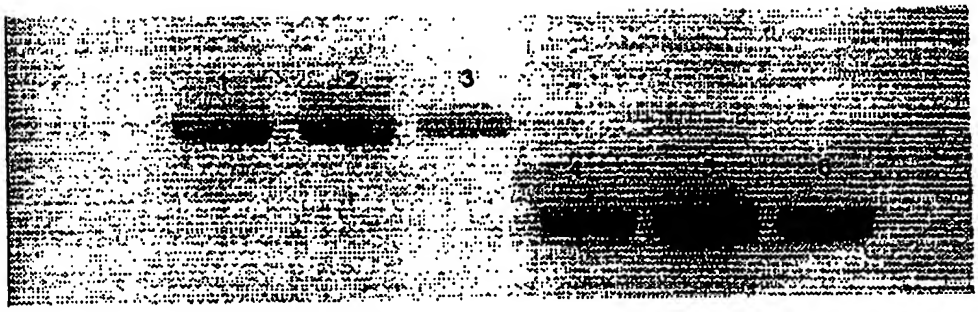
To obtain treated blood, the selected aliquot, in a sterile, UV-transmissive container, was treated simultaneously with a gaseous oxygen/ozone mixture and ultraviolet light at elevated temperature using an apparatus as generally described in aforementioned U.S. Patent No. 4,968,483 Mueller et.al. Specifically, 12 ml of citrated blood was transferred to a sterile, low density polyethylene vessel (more specifically, a Vasogen VC7002 Blood Container) for ex vivo treatment with stressors according to the invention. Using an apparatus as described in the aforementioned Mueller patent (more specifically, a Vasogen VC7001 apparatus), the blood was heated to 42.5±1°C and at that temperature irradiated with UV light principally at a wavelength of 253.7 nm, while oxygen/ozone gas mixture was bubbled through the blood to provide the oxidative environment and to facilitate exposure of the blood to UV. The constitution of the gas mixture was 14.5 ± 1.0 μg ozone/ml, with the remainder of the mixture comprising medical grade oxygen. The gas mixture was bubbled through the aliquot at a rate of 240 ± 24 ml/min for a period of 3 minutes.

There were 4 groups of Balb-C mice. The first, control group A-1 received no treatment. The second, control group B-1, was treated with physiological saline, 50 µl. The third, control group C-1, was sham treated, with 50 µl of blood which had been extracted but not treated with the stressors. The fourth, test group D-1, was treated with 50 µl of blood subjected to stressors as described above. Treatments, each involving intramuscular injection of 50 µl of the respective liquid, started on day 1, and was repeated every day for a total of 6 days.

The experiment was run in parallel to a test for contact hypersensitivity resistance in the mice, as described in applicants co-pending international patent application PCT/CA00/00433, so that the various groups had been pre-sensitized with dinitrofluorobenzene DNFB and were subsequently challenged, 24 hours after the last injection, with DNFB as described therein, but this is not a factor in the tests demonstrating the present invention.

Lymph nodes were drained from each of the animals after the conclusion of the procedures, and the lymph tissue tested by standard, known rtPCR technology, for expression of cytokine IFN-γ. The process of testing and analysis followed the procedures described by Kondo, S., et.al, (1996) J. Immunology, p.157: 482. Thus the PCR products were determined by scanning of photonegatives using a laser densitometer, and the densitometric value of the IFN-γ was normalized to that of the housekeeping gene β-actin. The analyses indicated that animals which had received a course of injection of blood subjected to stressors as described had significantly reduced IFN-γ contents in the fluid, as compared with controls and sham treated animals, as illustrated in the accompanying Figure. This indicates a marked reduction in protective IFN-γ cytokine secretion, which when applied to mammals, including humans, carrying CD5+ malignant B cells will down regulate the normal, prolific secretion of IFn-γ and lend them more susceptible to apoptosis such as that induced by chemotherapy and radiation treatment to attack the malignant cells.





1 - B-actin (VAS972)
2- B-actin (sham)
3 - B-actin (control

4- IFN-γ (VAS972)
5 -IFN-γ (sham)
6 -IFN-γ (control)

FIGURE 1